Influence of capacitation and fluids from the male and female genital tract on the zona binding ability of bull spermatozoa

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Before fertilization, inseminated spermatozoa acquire the ability to fertilize an egg by means of capacitation. Bovine sperm capacitation is influenced by factors originating from both the male and female genital tract, and results in intracellular and membrane changes of the spermatozoa that facilitate the induction of the acrosome reaction. However, the effects of reproductive tract secretions and capacitation on the binding of spermatozoa to the zona pellucida have not been investigated. In this study, a sperm–egg binding assay was used to determine whether the ability of bull spermatozoa to bind to the zona pellucida was altered during in vitro capacitation by heparin or oviductal fluid, or by treatment of spermatozoa from the cauda epididymidis with accessory sex gland fluid. In addition, biotinylated solubilized zona pellucida proteins were used to visualize zona binding on spermatozoa. The ability of bull spermatozoa to bind to the zona pellucida was increased after both heparin and oviductal fluid induced in vitro capacitation. Exposure of spermatozoa from the cauda epididymidis to accessory sex gland fluid resulted in a direct increase in zona binding ability, followed by a further increase during capacitation in vitro. Binding of solubilized zona proteins was restricted to the acrosomal cap of bull spermatozoa. It is suggested that the observed increased ability of bull spermatozoa to bind to the zona pellucida enables optimal sperm–egg attachment, which also relates to the induction of the acrosome reaction by the zona pellucida. Thus, increased zona binding ability is likely to be an essential part of the process of capacitation.

Introduction

After insemination, mammalian spermatozoa reside in the female reproductive tract for several hours before they acquire the ability to fertilize an egg by means of capacitation (Austin, 1951; Chang, 1951). Sperm capacitation involves a series of molecular and cellular changes, including changes in the concentration of intracellular ions, the adenylate cyclase cAMP system and the plasma membrane (for review, see Yanagimachi, 1994). Although the precise role these changes play in capacitation has not yet been clarified, they may facilitate two functions that appear to be essential for fertilization: the acrosome reaction and hyperactivated motility (for review, see Yanagimachi, 1994).

The mammalian oviduct provides the environment for capacitation of spermatozoa. In cattle, the oviductal isthmus may serve as a sperm reservoir, which has also been suggested as the site where capacitation occurs (First and Parrish, 1987). Heparin-like glycosaminoglycans and the oestrus-associated protein, which originate from the genital tract of cows, have been shown to stimulate capacitation in vitro (Parrish et al., 1989; McNutt and Killian, 1991; Anderson and Killian, 1994; King et al., 1994; Parrish et al., 1994). In addition, it has been shown that the effects of oviductal secretions on sperm function vary with the stage of the oestrous cycle and oviductal region (Anderson and Killian, 1994; Grippo et al., 1995).

Capacitation of bovine spermatozoa in vitro using heparin facilitates induction of the acrosome reaction by solubilized zona pellucida (ZP) proteins or by the fusogenic lipid lysophosphatidylcholine (LPC) (Florman and First, 1988a; Parrish et al., 1988). Although capacitation of bovine spermatozoa by heparin and oviductal secretions appears similar and involves increases in both internal Ca2+ and pH, differences have also been reported (Parrish et al., 1994; Vredenburgh-Wilberg and Parrish, 1995; King et al., 1994).

It has been shown that bull spermatozoa from the cauda epididymidis already possess the ability to fertilize an egg (Amann and Griep, 1974). However, accessory sex gland fluid do augment the fertility of cauda epididymal bull spermatozoa (Henault et al., 1995). In addition, both stimulatory and inhibitory factors originating from the bull genital tract have been shown to influence capacitation and
the susceptibility for acrosomal exocytosis in response to solubilized zona pellucida proteins or LPC (Florman and First, 1988; Miller et al., 1990; Therien et al., 1995).

Although several studies have demonstrated that the ability of bull spermatozoa to undergo the acrosome reaction in response to zona pellucida proteins or LPC is altered during capacitation and is influenced by both male and female genital tract fluids, it has not been reported whether the ability of spermatozoa to bind to the zona pellucida is also affected by capacitation. It is hypothesized that, during capacitation, the ability of bovine spermatozoa to bind to the zona pellucida is increased. Therefore, the purpose of this study was to determine whether the ability of bovine spermatozoa to bind to the zona pellucida is changed during in vitro capacitation, and whether this ability is influenced by accessory sex gland fluid.

Materials and Methods

Materials

All chemicals were of analytical grade and were purchased from Sigma (St Louis, MO) unless otherwise stated.

Experimental approach

Zona binding ability was studied by calculating the average number of spermatozoa that attached to a bovine zona pellucida in a sperm-egg binding assay. Solubilized biotinylated zona pellucida proteins were used to study the binding of zona pellucida proteins to individual spermatozoa. The fusogenic lipid LPC was used to induce the acrosomal reaction in capacitated spermatozoa (Parrish et al., 1988).

Ejaculates semen from three bulls was pooled, and aliquots were incubated in the following to determine whether zona binding ability was affected by in vitro capacitation in heparin or oviductal fluid: (1) modified Tyrode's medium (MTM; Parrish et al., 1988); (2) MTM supplemented with heparin (10 µg ml⁻¹); (3) 50% amplyary non-luteal oviductal fluid in MTM; or (4) 50% 50% ischemic non-luteal oviductal fluid in MTM. The concentration of oviductal fluid in MTM was based on a study by Grippi et al. (1995). At 0 h and 4 h of incubation, the zona binding ability of spermatozoa for each treatment was determined and the LPC assay was performed to assess capacitation. The experiment was replicated on four different days.

The zona binding ability of spermatozoa from the cauda epididymidis was compared with spermatozoa from the same collection that were exposed to accessory sex gland fluid to determine whether accessory sex gland fluid affected zona binding ability. At 0 h and 4 h of incubation in medium containing heparin, evaluations were made to assess capacitation and zona binding ability for both treatments. This experiment was replicated on two different days.

Isolation of bovine oocytes and zonae pellucidae

Ovaries from 26-week-old heifers were collected at an abattoir and stored at -80°C until the day of use. The oocytes were isolated by the procedure of Dunbar et al. (1980) with some modifications, as described in detail by Topper et al. (1997). Bovine oocytes were homogenized in a Glass Potter homogenizer and the zona pellucida fragments were separated from oocyte cell components by collecting the zona fragments on a 45 mm screen. This preparation was thoroughly washed with distilled water and resuspended in 50 mmol NH₄HCO₃ l⁻¹ (pH 7.2) with adjustment to 1 g SDS l⁻¹ and incubated for 30 min at 70°C. The solution was extensively dialysed against urea (7 mol l⁻¹) and then distilled water to remove SDS. This zona pellucida protein preparation was lyophilized and stored at 4°C.

Biotinylation of solubilized bovine zona pellucida proteins

Biotinylation of solubilized bovine zona pellucida proteins was accomplished in a 100 mmol sodium borate buffer 1⁻¹ (pH 8.8) by adding 0.2 mg sulfosuccinimidyl-6-(biotinamido)hexanoate (NHS-LC-biotin; Pierce, Rockford, IL) mg⁻¹ zona pellucida protein. After 4 h incubation at room temperature, the reaction was quenched with NH₄Cl, and unbound biotin was removed by washing the solution four times with PBS in a Centricon-10 centrifuge tube (Amicon, Beverly, MA) at 1000 g for 30 min. The protein concentration was determined using ovalbumin as a standard (Bradford, 1976). The biotinylated zona pellucida proteins were stored at -80°C.

Collection of oviductal fluid

Laparotomies were performed on cows to cannulate oviducts as described in detail by Kavanagh et al. (1992). Each oviduct contained ampullary and isthmic catheters that were exteriorized through flank incisions to sterile collection vials. The vials were retained in a canvas pouch mounted with adhesive to the flank. Vials containing oviductal fluid were replaced each day and stored in liquid nitrogen until use. Serum progesterone concentrations were measured each day and used to define the stage of the oestrous cycle (Killian et al., 1989). The non-luteal phase included those days for which serum progesterone concentrations were ≤ 1.5 ng ml⁻¹. Samples taken each day of ampullary non-luteal and isthmic non-luteal oviductal fluid were pooled and assessed for protein concentration.

Collection of epididymal spermatozoa and accessory sex gland fluids

The vasa deferentia of two bulls were cannulated under halothane anaesthesia using the method of Henault et al. (1995) to obtain spermatozoa from the cauda epididymidis. Spermatozoa were collected from indwelling vasa deferentia catheters after ejaculation, and accessory sex gland fluid was collected using an artificial vagina. After surgery, the accessory sex gland fluid was not used experimentally until it was free from spermatozoa. Accessory sex gland fluids from two bulls were pooled and centrifuged at 2400 g for 15 min.
Sperm preparation and capacitation

Semen was collected from three mature Holstein bulls via an artificial vagina. One millilitre of semen from each bull was pooled and spermatozoa were washed twice (for 10 min at 500 g) with sterilized protein-free MTM. Washed spermatozoa were incubated for 4 h at a concentration of 5 x 10^7 ml^-1 in: (1) MTM (containing 6 mg ml^-1 BSA); (2) MTM supplemented with heparin (10 mg ml^-1) (MTMH); (3) 50% amnillary non-luteal oviductal fluid in MTM (ANL) (v/v); or (4) 50% isithmic non-luteal oviductal fluid in MTM (INAL) (v/v). All treatments were incubated at 39°C (5% CO2 in air) for 4 h to allow capacitation of spermatozoa. Spermatozoa from the cauda epididymides of two bulls were collected and either resuspended 1:5 (v/v) in MTM or accessory sex gland fluid. The spermatozoa were incubated for 15 min at 39°C. After incubation, the spermatozoa from both treatments were washed twice (for 10 min at 500 g) with protein-free MTM, and resuspended at a concentration of 5 x 10^7 ml^-1 in MTM or MTMH, as described for ejaculated spermatozoa.

Lysophosphatidylcholine assay

After 0 h and 4 h of incubation, the fusogenic lipid LPC was used to induce the acrosome reaction in capacitated spermatozoa (Parrish et al., 1988). Briefly, a 100 µl aliquot of spermatozoa was incubated for 10 min at 39°C with 12.5 µl BSA (50 mg ml^-1; Fraction V) and 16.2 µl stock solution of LPC (60 mg ml^-1, purified from egg yolk). Spermatozoa were stained with eosin-aniline blue and the percentage of spermatozoa that were acrosome-reacted was assessed by differential interference contrast microscopy (Way et al., 1995).

Binding of solubilized biotinylated zona pellucida proteins to bull spermatozoa

At 0 h and 4 h of incubation of the spermatozoa, 125 µl sperm suspension was incubated with solubilized biotinylated zona pellucida proteins (5 µg ml^-1, 39°C, 15 min). The spermatozoa were then washed twice (5 min, 500 g) with protein-free MTM, and fixed with 11 g glutaraldehyde l^-1 in PBS. Biotinylated BSA was used as a negative control.

The binding of zona pellucida proteins on the sperm surface was visualized using the method of Yurewicz et al. (1993). Fixed spermatozoa were centrifuged and resuspended in 200 µl streptavidin–alkaline phosphatase (streptavidin–AP; 1 µg ml^-1) prepared in PBS. After a 60 min incubation at room temperature, spermatozoa were washed twice and the volume was adjusted to 50 µl with PBS. The spermatozoa were reacted with a mixture containing 0.3 mg ml^-1 p-nitroblue tetrazolium chloride, 0.15 mg ml^-1 5-bromo-4-chloro-3-indolyl phosphate in 5 mmol sodium carbonate buffer l^-1, pH 9.8. After 30 min incubation at room temperature, spermatozoa were washed twice with PBS. Differential interference contrast microscopy was used to assess the acrosomal status of the spermatozoa and to determine whether individual spermatozoa had bound solubilized zona pellucida proteins. One hundred spermatozoa from each treatment were examined for zona pellucida-binding pattern and acrosomal status.

Sperm–egg binding assay

Sperm–egg binding assays were carried out using the method of Way et al. (1997) with slight modifications. Briefly, 50 oocytes per treatment were thawed at room temperature, transferred from storage medium and washed in protein-free MTM. The oocytes were placed in groups of ten in 50 µl drops containing protein-free MTM. At 0 h and 4 h of incubation of the spermatozoa, the oocytes were inseminated with 10^5 spermatozoa and incubated for 15 min at 39°C (5% CO2 in air). Oocytes were washed once with protein-free MTM and fixed in 11 g glutaraldehyde l^-1 in PBS. The oocytes were placed on a slide, and a coverslip, mounted at each corner with a mixture of paraffin wax and petroleum jelly, was placed and gently lowered until the zona pellucida of each oocyte ruptured. Zonae pellucidae were rinsed with PBS to remove the contents of the oocytes and spermatozoa were stained with Hoechst 33342 to determine the number of spermatozoa attached.

Statistical analysis

Data on the number of spermatozoa that attached to each egg were analysed as over-dispersed Poisson counts with a generalized linear mixed model (Engel and Keen, 1994). A logarithmic link function was specified; that is, fixed and random effects were introduced on the log scale. Fixed effects in the model were main effects for treatments, time and days, and interaction effects between days and times and treatments and days. Random effects in the model were interactions between days and treatments, between days, treatments and times, and droplet effects. This amounted to three components of variance in addition to a multiplicative overdispersion factor.

Data on the percentage of spermatozoa that showed binding of solubilized biotinylated zona pellucida protein were initially analysed with a generalized linear model (GLM) (McCullagh and Nelder, 1989) with a probit link function. Main effects for treatments, days and time, and interaction between treatments and days and between treatments and time were considered. The analysis showed that observations were over-dispersed with respect to the binomial variance function. Subsequently, overdispersion was introduced through residual random effects on the probit scale. The final analysis was performed with a GLM (Engel and Keen, 1994; Engel et al., 1995; Engel and Buist, 1996). Interactions between days and treatments were entered as random effects in the model.

Data on the percentage of LPC-induced, acrosome-reacted spermatozoa were analysed like the data on the percentage of spermatozoa that bound solubilized zona pellucida proteins; that is, using a GLM with a probit link and residual random effects to account for over-dispersion.

All calculations were performed with Genstat 5 (1993). A P value ≤ 0.05 was considered statistically significant.
The percentage of acrosome-reacted spermatozoa after exposure to LPC was significantly higher after 4 h of incubation in MTM and INL oviductal fluid than at 0 h of incubation (Fig. 4). Pairwise comparisons with the control showed that, at 0 h of incubation, there were no significant differences among treatments. At 4 h of incubation, the percentage of acrosome-reacted spermatozoa in the control was significantly less than it was for the spermatozoa that had been incubated in MTMH or INL oviductal fluid. The difference between the percentage of acrosome-reacted spermatozoa in the MTMH and INL treatment groups was not significant. The value of the percentage of acrosome-reacted spermatozoa given ANL treatment was in between the values for the MTMH and INL treatment groups, and the control.

Exposure of spermatozoa from the cauda epididymis to accessory sex gland fluid increased the average number of spermatozoa that attached to the zona pellucida (Fig. 5). This increase was already appreciable immediately after spermatozoa from the cauda epididymis had been exposed to accessory sex gland fluid at 0 h of incubation. Moreover, after 4 h of incubation in MTMH, a further increase in the average number of spermatozoa that bound to the zona pellucida was only observed after spermatozoa from the cauda epididymis had been exposed to accessory sex gland fluid.

Addition of accessory sex gland fluid to spermatozoa from the cauda epididymis also had an effect on the percentage of spermatozoa that had bound solubilized zona pellucida proteins at the apical ridge or at the acrosomal cap. After exposure to accessory sex gland fluid at 0 h of incubation, the percentage of spermatozoa from the cauda epididymis that had bound solubilized zona pellucida proteins was increased markedly (Fig. 6). The percentage of spermatozoa that showed binding of zona pellucida proteins appeared to increase during incubation in MTMH, but this was not significant.

**Discussion**

On the basis of evidence from a variety of microscopic and biochemical studies, it is generally accepted that the sperm reacted spermatozoa did not show binding of biotinylated zona pellucida proteins. Binding studies using biotinylated BSA as a control showed that BSA did not bind preferentially to specific parts of the spermatozoa, but exhibited faint non-specific background labelling.

After 4 h of incubation of bovine spermatozoa in MTM supplemented with either heparin, 50% ampullary oviductal fluid, or 50% isthmic oviductal fluid, the percentage of spermatozoa that had bound solubilized zona pellucida proteins at the apical ridge (Fig. 2a) or over the entire acrosomal cap (Fig. 2c) was significantly greater than it was at 0 h of incubation (Fig. 3). The percentages of spermatozoa at 0 h and 4 h of incubation in the control medium (MTM only) were not significantly different. Pairwise comparisons between the MTMH, INL and ANL treatments with MTM at 4 h of incubation showed that the percentage of spermatozoa that bound solubilized bovine zona pellucida proteins was significantly greater for the MTMH and INL groups, whereas for spermatozoa that were incubated in ANL oviductal fluid, the difference with MTM was not significant (0.05 < P < 0.10).

Incubation of bovine spermatozoa for 4 h in modified Tyrode's medium supplemented with heparin (MTMH), 50% ampullary oviductal fluid (ANL), or 50% isthmic oviductal fluid (INL), resulted in significantly more spermatozoa attached to the zona pellucida, than in control medium, that is, MTM only (Fig. 1). At 0 h of incubation, pairwise comparisons between treatments showed no significant differences. After 4 h of incubation, the average number of attached spermatozoa for the MTMH and ANL treatments were not significantly different, but the average numbers of attached spermatozoa for these treatments were significantly less than they were in the INL treatment (Fig. 1).

Differential interference microscopic evaluations of spermatozoa that were exposed to solubilized biotinylated zona pellucida proteins revealed spermatozoa that appeared as: (1) zona pellucida protein negative, acrosome-intact (Fig. 2a); (2) zona pellucida protein binding at the apical ridge, acrosome-intact (Fig. 2b); (3) zona pellucida protein binding at the acrosomal cap, acrosome-intact (Fig. 2c); or (4) zona pellucida protein negative, lost acrosome (Fig. 2d). Occasionally, labelled zona pellucida proteins bound to the equatorial region of spermatozoa, presumably associated with remnants of the acrosomal membranes. Acrosome-
surface is continuously modified during capacitation. Lectins have been a particularly helpful tool with which to map sperm membrane glycoproteins and glycolipids and to monitor the changes in their distribution during capacitation (Cross and Overstreet, 1987; Lee and Ahuja, 1987; Mahmoud and Parrish, 1992, 1996; Ashworth et al., 1995). Antibodies have also proven to be useful probes for studying the dynamics of sperm surface proteins during capacitation (Fusi et al., 1992; Ambrose et al., 1993; Rajamahendran et al., 1994). However, the precise physiological implication of many of these observed changes is not entirely clear. The approach used in the present study, using biotinylated solubilized zona proteins, enabled surface changes of bull spermatozoa during in vitro capacitation, which may directly relate to the ability of spermatozoa to bind to the zona pellucida, to be monitored.

The present study showed that during capacitation in vitro, induced by heparin or oviductal fluid, the ability of bovine spermatozoa to bind to the zona pellucida is increased. In addition, the ability of bull spermatozoa to bind to the zona pellucida was shown to be influenced by accessory sex gland fluid. The increased ability of
spermatozoa to bind to the zona pellucida after capacitation in vitro is matched by an increased ability of the spermatozoa to bind solubilized zona pellucida proteins.

The use of these solubilized zona proteins enabled the visualization and localization of the zona binding site on the sperm surface, and the inspection of the acrosomal status of the spermatozoa with increased zona binding ability. Differential interference microscopy indicated that most spermatozoa that bound solubilized zona pellucida proteins were acrosome-intact. Therefore, we conclude tentatively that the observed binding of solubilized zona pellucida proteins in the majority of the spermatozoa takes place at the surface of the intact sperm plasma membrane. However, according to Nolan et al. (1992), spermatozoa exhibiting an initial stage of acrosomal vesiculation appear acrosome-intact by phase and differential interference contrast microscopy. The observation that a minority of the spermatozoa that bound solubilized zona pellucida proteins had a somewhat fuzzy, rippled or swollen acrosome, perhaps reflects such an initial stage of acrosomal exocytosis, which in the present study could have been induced by the solubilized zona proteins to which the spermatozoa had been exposed.

The present study does not provide direct evidence for the mechanism of the increased zona binding ability of bovine spermatozoa during capacitation. However, recent studies suggest that binding sites for the lectin WGA on bovine spermatozoa are either structurally altered or detached during heparin-induced capacitation (Medeiros and Parrish, 1991; Mahmoud and Parrish, 1992, 1996). Loss of sialic acid from the sperm surface leads to a decrease in the net negative surface charge of spermatozoa (Langlais and Roberts, 1985). Iqbal and Hunter (1995) used electrophoresis to show that, after the capacitation of bovine spermatozoa, net negative surface charge was decreased. Since the binding between spermatozoa and the zona pellucida involves hydrophobic as well as ionic interactions (Urch and Patel, 1991; Naz and Ahmad, 1994), the decreased net negative surface charge that takes place during capacitation may facilitate the binding of bovine zona pellucida proteins to the spermatozoa.

A second mechanism that could be involved in the increase of the zona binding ability during capacitation is that changes in fluidity and permeability of the sperm plasma membrane take place during capacitation (for review, see Langlais and Roberts, 1985), which result in the...
migration and reorganization of both lipids and proteins. Therien et al. (1995) elaborated on this model of membrane modulation and suggested that heparin or heparin-like glycosaminoglycans, known to be present in oviductal fluid (Lee and Ax, 1984), stimulate membrane reorganization through their interaction with sperm-bound bovine seminal plasma (BSP) proteins that have been described to bind to membrane phospholipids (Therien et al., 1995). The fact that both heparin and factors from the accessory sex glands were involved in the changes in zona binding ability of the spermatozoa, shown in the present study, suggests that the above mechanisms of membrane modulation are responsible for the observed changes in zona-binding ability. For example, it could be envisaged that the changes in membrane reorganization and the changes in surface charge, described above, lead to exposure, activation or increased affinity of already existing binding sites in the sperm plasma membrane. However, at present, no zona pellucida-binding molecules have been identified for bovine spermatozoa.

Parrish et al. (1988, 1989) showed the potentiating effect of both heparin and oviductal fluid on LPC-induced acrosome reactions. In the present study, LPC-induced acrosome reactions were determined to assess the capacitation of spermatozoa. The percentage of LPC-induced acrosome reactions was indeed higher after the incubation of spermatozoa in both heparin or oviductal fluid, indicating capacitation had occurred. These results do not reveal the exact relation and time-frame between an increased zona binding ability and an increased susceptibility to induction of the acrosome reaction. Further research is required to uncover details of this relation.

The results of the sperm–egg binding assay support the reports of Anderson and Killian (1994) and Grippo et al. (1995) that the effects of oviductal fluid on sperm function are different at different regions of the oviduct. The zona binding ability of bovine spermatozoa in the present study was greater after 4 h of incubation in isthmic oviductal fluid than after incubation in ampullary oviductal fluid. This finding supports the concept of the oviductal isthmus that provides for a population of capacitated spermatozoa at the site of fertilization (First and Parrish, 1987).

The results of the present study indicate that accessory sex gland fluids play a role in the increase of the zona binding ability of bull spermatozoa during capacitation. When accessory sex gland fluid had been added, a direct increase of zona binding ability as well as a stimulation of the increase of zona binding ability during capacitation in vitro was observed. As stated above, the function of factors from the accessory glands could be to induce or facilitate membrane reorganization leading to changes in the zona-binding ability of the spermatozoa. Desnoyers and Manjunath (1992) proposed that BSP proteins originating from seminal vesicles play a regulatory role during capacitation. Therien et al. (1995) showed that BSP proteins bind to membrane

Fig. 5. Effect of accessory sex gland fluid on the average number of attached spermatozoa from the cauda epididymis per oocyte in a sperm–egg binding assay: spermatozoa from the cauda epididymis (CS); spermatozoa from the cauda epididymis that were mixed with accessory sex gland fluid (CSA) [●, time = 0 h; □, time = 4 h]. Different numbers of asterisks indicate significant differences in mean number of attached spermatozoa per oocyte ($P \leq 0.05$).

Fig. 6. Effect of accessory sex gland fluid on the percentage of spermatozoa from the cauda epididymis that showed binding of solubilized biotinylated zona pellucida proteins (5 μg ml$^{-1}$; 15 min): spermatozoa from the cauda epididymis (CS); spermatozoa from the cauda epididymis that were mixed with accessory sex gland fluid (CSA) [●, time = 0 h; □, time = 4 h]. Asterisks indicate significant differences in percentage of spermatozoa that underwent the acrosome reaction ($P \leq 0.05$).
phospholipids and may be involved in membrane reorganization. In addition, BSP proteins reduced the time required for solubilized zona pellucida proteins and LPC to induce the acrosome reaction (Manjunath et al., 1994; Therien et al., 1995).

In pigs, a regulatory role during capacitation has been described for the spermadhesins that originate from the cauda epididymis or the accessory sex glands (Dostalova et al., 1994). Spermadhesins in pigs have also been shown to bind zona pellucida proteins and, therefore, could be involved in sperm-egg attachment (Dostalova et al., 1994). However, it remains to be established whether the spermadhesins are present on the small population of spermatozoa that actually meets the egg in vivo (Töpper-Petersen, 1996).

The direct stimulation of the binding of spermatozoa from the cauda epididymidis to the zona pellucida upon addition of accessory sex gland fluid means that the membrane changes that are assumed to be involved, are accomplished very quickly in a subtraction of the spermatozoa. However, putative spermadhesin-like factors that bind to the zona pellucida (see Dostalova et al., 1994) may have contributed to this initial increase in binding of spermatozoa from the cauda epididymidis to the zona pellucida.

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References


Arimbozo JD, Rajamahendran R and Lee CYG (1993) Monoclonal antibodies HS-9 and HS-11 as potential markers to study bovine sperm capacitation Theriogenology 39 178


Austin CR (1951) Observations on the penetration of the sperm into the mammalian egg Australian Journal of Scientific Research 4 581–596


Chang MC (1951) Fertilizing capacity of spermatozoa deposited into the Fallopian tubes Nature 168 697–798


Dostalova Z, Calvete JJ, Sana I and Topper-Petersen E (1994) Quantitation of boar spermadhesins in accessory gland fluids and on the surface of epididymyal, ejaculated and capacitated sperm Biochimica Biophysica Acta 1200 48–54


Flomman HM and First NL (1988b) Regulation of acrosomal exocytosis II. The zona pellucida-induced acrosome reaction of bovine spermatozoa is controlled by extrinsic positive regulatory elements Developmental Biology 128 464–473


Iqbal N and Hunter AG (1995) Comparison of various bovine sperm capacitation systems for their ability to alter the net negative surface charge of spermatozoa Journal of Dairy Science 78 84–90


Mahmoud AI and Parrish JJ (1992) Flow cytometric analysis of lectin binding to bovine sperm during capacitation with heparin or oviduct fluid Theriogenology 37(1) 252


Medeiros CO and Parrish JJ (1991) Changes in Triticum vulgaris (WGA) binding to bovine sperm during capacitation Journal of Animal Science 69 (Supplement 1) 460

Miller DJ, Winer MA and Ax RL (1990) Heparin-binding proteins from seminal plasma bind to bovine spermatozoa and modulate capacitation by heparin Biology of Reproduction 42 899–915


Capacitation of bovine spermatozoa by oviduct fluid Biology of Reproduction 40 1020–1025
Parrish JJ, Susko-Parrish JL, Uguz C and First NL (1994) Differences in the role of cyclic adenosine 3',5'-monophosphate during capacitation of bovine sperm by heparin or oviduct fluid Biology of Reproduction 51 1099–1108
Töpfer-Petersen (1996) Molecular mechanism of fertilization in the pig Reproduction in Domestic Animals 31 93–100
Urch UA and Patel H (1991) The interaction of boar sperm acrosin with its natural substrate, the zona pellucida and with polysulfated polysaccharides Development 111 1165–1172
Yurewicz EC, Pack BA, Armant DR and Sacco AG (1993) Porcine zona pellucida ZP3a glycoprotein mediates binding of the biotin-labelled Mr 55000 family (ZP3) to boar sperm membrane vesicles Molecular Reproduction and Development 36 382–389